R E M A R K S

Claim For Priority Under 35 USC 119

On page 1, item 12 a) of the July 29, 1008 Office Action, the box "All" should have included an "X", not the box "Some". It is noted that there is one Japanese priority application (JP 2002-250223).

Information Disclosure Statement

On the copy of sheet 2 of the May 16, 2005 IDS Form returned with the July 29, 2008 Office Action, the Examiner drew a line through JP 2726672. However, JP 2726672 should have been considered and made of record, since it is a related family member of USP 4,952,581.

Claim Amendments and New Claims

The amendments to the claims involving "salts or esters thereof" are supported in the paragraph bridging pages 3 and 4 and the first full paragraph on page 4 of the originally filed specification.

The amendments to claims 1 and 2 regarding "optionally a pharmaceutically carrier thereof" is supported in the specification on page 7, lines 6 to 18.

New claims 21 and 22 are supported by Table 1 on page 14 of the originally filed specification.

Abstract

The Abstract was amended hereinabove to make editorial revisions.

Objection to the Specification

The specification was objected to for the reasons set forth in item no. 7 at the top of page 3 of the July 29, 2008 Office Action. The Examiner required the filing of a substitute specification in "proper idiomatic English."

A clean version of a SUBSTITUTE SPECIFICATION and a marked-up copy of the specification showing the changes are submitted concomitantly herewith.

The SUBSTITUTE SPECIFICATION does not introduce any new matter.

Withdrawal of the objection to the specification is respectfully requested.

Objections to the Drawings

The drawings were objected to for the reasons indicated in item no. 8 bridging pages 3 and 4 of the July 29, 2008 Office Action. The Examiner alleged that Figures 1 and 3 refer to "Compound A" and Figures 2 and 4 refer to "Compound B." This is incorrect. Figures 1 to 4 do not refer to "Compound A" or "Compound B", rather the Brief Description of the Drawings on pages 8 to 9 of the specification refers to "Compound A" and "Compound B."

The Examiner required that corrected drawing sheets be provided or the specification be amended to define "Compound A" and "Compound B."

The enclosed SUBSTITUTE SPECIFICATION includes a revision to the Brief Description of the Drawings to define "Compound A" and

"Compound B" to include the definitions of these compounds, as stated on page 11, lines 6 to 8 of the originally filed specification.

Withdrawal of the objection to the Drawings is respectfully requested.

Obviousness Rejection Under 35 USC 103

Claims 1 to 4 and 13 to 16 were rejected under 35 USC 103 as being unpatentable over EP 286903 ("Bito") in view of USP 7,015,210 to Aiken, P. Vasantha Rao et al., *Modulation of Aqueous Humor Outflow Facility by the Rho Kinase-Specific Inhibitor Y-27632*, 42, INV. OPHTHALMOL. VIS. SCI., 1029-1037, (April 2001) and USP 6,271,224 to Kapin et al. for the reasons set forth in item nos. 9 to 14 on pages 4 to 6 of the July 29, 2008 Office Action.

It was admitted in the July 29, 2008 Office Action that Bito et al. do not recite any of applicants' claimed prostaglandins or Rho-kinase inhibitors as effective combination therapeutics.

As pointed out by the Examiner, Bito et al. describe that combination therapy is useful for medical treatment of glaucoma; that regulating both the production and outflow of aqueous humor are effective in controlling intraocular pressure ("IOP"); that there are two aqueous humor outflow pathways, i.e., an uveoscleral outflow pathway and a trabecular meshwork outflow pathway; and that prostaglandin ("PG") derivatives reduce IOP by increasing uveoscleral outflow.

Bito requires the presence of an adrenergic blocking agent, which is not called for in applicants' composition claims.

Bito describes in columns 2 to 3 that although pilocarpine, a drug to promote the aqueous humor outflow through the trabecular meshwork outflow pathway, works effectively in combination with an adrenergic blocking agent, since pilocarpine decreases the aqueous humor outflow through the uveoscleral outflow pathway, pilocarpine may have an adverse effect, rather than a beneficial effect. Furthermore, Bito et al. teach in the paragraph bridging columns 2 to 3 that an adrenergic blocking agent has to be proved not to block the ocular hypotensive effect

of a prostaglandin. These disclosures by Bito et al. mean that unless the combination of the drugs is actually tested, a person or ordinary skill in the art would not know whether beneficial or adverse ocular hypotensive results would be obtained.

Bito describe in his examples the combination of timolol, betaxolol, and levobunalol with $PGF_{2\alpha}$-1-isopropyl ester, i.e., the combination of a drug to control the aqueous humor production with a drug to promote the uveoscleral outflow of aqueous humor, and the advantageous resulst thereof. Bito does not describe in his examples a combination of drugs, wherein each drug promotes the aqueous humor outflow and the advantageous results thereof.

Aiken describes that prostaglandins promoting the aqueous humor outflow and showing an IOP lowering effect, such as latanoprost, unoprostone isopropyl and travaprost, can be used as part of a combination therapy when paired with an epoxy-steroidal aldosterone receptor antagonist (an agent to control aqueous humor production). Aiken also describes that the combined use of an aqueous humor production controlling agent and an aqueous humor outflow promoting agent is preferable. Aiken does not

describe at all any specific examples of such a combination. Moreover, since an epoxy-steroidal aldosterone receptor antagonist is an essential component in Aiken (which is not called for in applicants' composition claims), an aqueous humor production controlling agent is an essential component in Aiken. There is no description or suggestion in Aiken of the combination of drugs, wherein each drug has an aqueous outflow promoting effect and the advantageous results thereof.

Rao et al. and Kapin et al. disclose merely the functions or properties of compounds described in the Examples of the present specification.

In contrast to the cited reference, the presently claimed invention involves a combination of drugs, wherein each drug promotes the aqueous humor outflow, and thus provides advantageous results for the use thereof. In other words, the presently claimed invention relates to a combination of (1) a Rho kinase inhibitor having a new action mechanism (promoting aqueous humor outflow by acting directly on the trabecular meshwork) and promoting the aqueous humor outflow through the trabecular

meshwork outflow pathway and (2) a prostaglandin, which promotes aqueous humor outflow through the uveoscleral outflow pathway.

Whereas Bito and Aiken describe the combination of an aqueous humor production controlling agent and an aqueous humor outflow promoting agent, and the advantageous results thereof, Bito and Aiken do not describe or suggest the combination of drugs, wherein each drug promotes the aqueous humor outflow and the advantageous results thereof.

More particularly, Bito and Aiken do not teach or suggest the combination of (1) a Rho kinase inhibitor having a new action mechanism which promotes the aqueous humor outflow through the trabecular meshwork outflow pathway and (2) a prostaglandin, which promotes the aqueous humor outflow through the uveoscleral outflow pathway. Furthermore, considering the disclosure in Bito that the combined use of the drugs may have an adverse effect, rather than a beneficial effect, a person of ordinary skill in the art would not be able to predict the advantageous results of the presently claimed invention.

In view of the above, it is respectfully submitted that one of ordinary skill in the art would not arrive at applicants' presently claimed invention based on the disclosure of the references.

Withdrawal of the 35 USC 103 rejection is therefore respectfully requested.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,

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(2) MARKED-UP VERSION OF THE SUBSTITUTE SPECIFICATION

MARKED-UP VERSION OF THE SUBSTITUTE SPECIFICATION

~~Specification~~

Therapeutic Agent for Glaucoma Comprising Rho Kinase Inhibitor and Prostaglandin

Technical Field

The present invention relates to a therapeutic agent for glaucoma comprising the combination of a Rho kinase inhibitor and a prostaglandin.

Background Art

Glaucoma is an intractable ocular disease with a risk of blindness, involving the increase of intraocular pressure due to various factors and by disordering internal tissues of eyeballs (retina, an optic nerve and the like). A general method of treating glaucoma is intraocular pressure reduction therapy, which is exemplified by pharmacotherapy, laser therapy, surgery therapy and the like.

For ~~the~~ pharmacotherapy, drugs such as sympathomimetic agents (nonselective stimulants such as epinephrine, α_2 stimulants such as apraclonidine), sympatholytic agents (β-blockers such as timolol and befunolol, α_1-blokers such as bunazosin hydrochloride), parasympathomimetic agents (pilocarpine and the like), carbonic anhydrase inhibitors (acetazolamide and the like) and prostaglandins (isopropyl unoprostone, latanoprost, travoprost, bimatoprost and the like) have been used.

Recently, a Rho kinase inhibitor was found to serve as a therapeutic agent for glaucoma based on a new mechanism of action (WO 00/09162). Invest. Ophthalmol. & Vis. Sci., 42 (1), 137-144 (2001) discloses that ~~the~~ a Rho kinase inhibitor increases the aqueous humor outflow from a trabecular meshwork outflow pathway thereby reducing intraocular pressure~~, and~~ . Invest. Ophthalmol. & Vis. Sci., 42 (1), 137-144 (2001) and Invest. Ophthalmol. & Vis. Sci., 42 (5), 1029-1037 (2001) suggest that the mechanism of action is reconstruction of cytoskeleton in trabecular meshwork cells.

Combined use of drugs having actions of reducing intraocular pressure to treat glaucoma has already been studied and there are some reports on the studies. For example, Japanese Patent No. 2726672 reports combined administration of the sympatholytic agent with prostaglandins. WO 02/38158 discloses a method of treating glaucoma by administering ~~some~~ to eyes a combination of drugs having ~~actions~~ a capacity of reducing intraocular pressure ~~in combination to eyes~~.

However, ~~any~~ such reports do not describe the Rho kinase inhibitor at all, and naturally, there is no description concerning advantageous effects brought about by combining the Rho kinase inhibitor with prostaglandins, either.

As mentioned above, ~~neither~~ there has been no study ~~nor~~ or report ~~has been made~~ concerning the therapeutic effects on glaucoma obtained by combining ~~the~~ a Rho kinase inhibitor with prostaglandins~~, so far~~.

~~Disclosure~~ Summary of the Invention

It ~~is a~~ was a very interesting ~~subject to find utility as~~ finding of the present inventors to discover a therapeutic agent for glaucoma ~~due to~~ comprising a combination of a Rho kinase inhibitor and a prostaglandin.

Studying precisely the effects due to the combination of a Rho kinase inhibitor and a prostaglandin, the present inventors found that an action of reducing intraocular pressure is increased and/or the persistence of the action is improved by combining these drugs compared with a case where each drug is used alone, and consequently the present inventors completed the present invention. Detailed test methods and their effects are described ~~later~~ hereinafter in the section ~~of~~ entitled "Pharmacological Tests". A remarkable increase in the action of reducing intraocular pressure and/or a remarkable improvement of the persistence of the action was observed by combining a Rho kinase inhibitor with a prostaglandin.

The present invention relates to a therapeutic agent for glaucoma comprising the combination of a Rho kinase inhibitor and a prostaglandin. These drugs each other complement and/or enhance their actions.

Detailed Description of the Invention

~~For the~~ Regarding the mode of administration ~~mode~~, each of the Rho kinase inhibitor and the prostaglandin can be in a separate preparation and these drugs can be administered in combination. Alternatively, these drugs can be formulated in a single preparation to be administered. In other words, these drugs can be administered in a mixture.

The Rho kinase inhibitors and prostaglandins ~~of~~ used in the present

invention include salts thereof. When these compounds have a basic group such as an amino group, they ~~can be~~ form salts with an inorganic acid such as hydrochloric acid or nitric acid or with an organic acid, such as ~~with~~ oxalic acid, succinic acid or acetic acid. When they have an acidic group such as a carboxyl group, they ~~can be~~ form salts with an alkali metal such as sodium or potassium or with an alkaline earth metal such as calcium.

The Rho kinase inhibitors and prostaglandins ~~of~~ used in the present invention include derivatives thereof such as esters. Specific examples of esters are alkyl esters such as methyl esters, ethyl esters and isopropyl esters.

The present invention is characterized by treating glaucoma with ~~the~~ a combination of a Rho kinase inhibitor and a prostaglandin.

The Rho kinase inhibitor used in the present invention means a compound which inhibits serine/threonine kinase activated ~~with~~ by the activation of Rho. Examples of Rho kinase inhibitors are ~~the~~ compounds which inhibit ROKα (ROCK-II), p160ROCK (ROKβ, ROCK-I) and other compounds which inhibit proteins having a serine/threonine kinase activity. Specific Rho kinase inhibitors are exemplified by Rho kinase inhibitors such as (R)-trans-N-(pyridin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide and (R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)-benzamide disclosed in WO 98/06433 and WO 00/09162; Rho kinase inhibitors such as 1-(5-isoquinolinesulfonyl)homopiperazine and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine disclosed in WO 97/23222 and Nature, 389, 990-994 (1997); Rho kinase inhibitors such as (1-benzylpyrrolidin-3-yl)-(1H-indazol-5-yl)amine disclosed in WO 01/56988; Rho kinase inhibitors such as (1-benzylpiperidin-4-yl)-(1H-indazol-5-yl)amine disclosed in WO 02/100833; Rho kinase

inhibitors such as N-[2-(4-fluorophenyl)-6,7-dimethoxy-4-quinazolinyl]-N-(1H-indazol-5-yl)amine disclosed in WO 02/076976~~,~~ ; Rho kinase inhibitors such as N-4-(1H-indazol-5-yl)-6,7-dimethoxy-N-2-pyridin-4-yl-quinazolin-2,4-diamine disclosed in WO 02/076977; and Rho kinase inhibitors such as 4-methyl-5-(2-methyl-[1,4]diazepan-1-sulfonyl)isoquinoline disclosed in WO 99/64011.

~~On the other hand, any prostaglandins~~ Any prostaglandin having the action of reducing intraocular pressure and which has utility in treating glaucoma can be used in the present invention. Prostaglandins having ~~the~~ an action of reducing intraocular pressure are specifically exemplified by prostaglandins described in Japanese Laid-open Patent Publication No. 1418/1984 (natural prostaglandins, particularly prostaglandin F2α)~~,~~ ; prostaglandins such as latanoprost as described in Published Japanese Translation of PCT No. 501025/1991~~,~~ ; prostaglandins such as isopropyl unoprostone as described in Japanese Laid-open Patent Publication No. 108/1990~~,~~ ; prostaglandins such as bimatoprost as described in Published Japanese Translation of PCT No. 501310/1996~~,~~ ; and prostaglandins such as travoprost as described in Japanese Laid-open Patent Publication No. 182465/1998. In particular, latanoprost, isopropyl unoprostone, bimatoprost ~~or~~ and travoprost, which ~~has~~ have already been on the market as a therapeutic agent of glaucoma, ~~is preferably used~~ are preferred for use in the present invention.

Examples of glaucoma in the present invention are primary open angle glaucoma, normal intraocular tension glaucoma, hypersecretion glaucoma, ocular hypertension, acute angle-closure glaucoma, chronic closed angle glaucoma, combined-mechanism glaucoma, corticosteroid glaucoma, amyloid

glaucoma, neovascular glaucoma, malignant glaucoma, capsular glaucoma, plateau iris syndrome and the like.

To carry out the present invention, preparations can be in the form of two preparations prepared by formulating a Rho kinase inhibitor and a prostaglandin separately or one preparation prepared by mixing these ingredients. Particular techniques are unnecessary for the formulation, and the preparations can be prepared using widely[[-]]used techniques. A preferred method of administration is ~~eye~~ topical eye administration, and a preferred dosage form is an ophthalmic solution or an eye ointment.

When a Rho kinase inhibitor and a prostaglandin are formulated in preparations separately, each preparation can be prepared according to known methods. For example, the Rho kinase inhibitor can be formulated in preparations by referring to the Formulation Examples described in the above-mentioned International Publications (WO 00/09162 and WO 97/23222). Prostaglandins can be formulated in preparations by referring to the Formulation Examples described in the above-mentioned Japanese Laid-open Patent Publications and Published Japanese Translations of PCT (i.e. Japanese Laid-open Patent Publication No. 1418/1984, Published Japanese Translation of PCT No. 501025/1991, Japanese Laid-open Patent Publication No. 108/1990, Published Japanese Translation of PCT No. 501310/1996 and Japanese Laid-open Patent Publication No. 182465/1998), and particularly for latanoprost, isopropyl unoprostone, bimatoprost, travoprost and the like, which have already been on the market as ~~the~~ therapeutic agents for glaucoma, commercially available preparations thereof can be used.

~~The~~ A formulation containing a Rho kinase inhibitor and a prostaglandin in a mixture also can be ~~also~~ prepared according to known methods. The ophthalmic solutions can be prepared, using isotonic agents such as sodium chloride and concentrated glycerin; buffers such as sodium phosphate buffer and sodium acetate buffer; surfactants such as polyoxyethylene sorbitan monooleate, stearate polyoxyl 40, and polyoxyethylene hardened castor oil; stabilizers such as sodium citrate and sodium edetate; and preservatives such as benzalkonium chloride and paraben, as needed. The pH should be within an ophthalmologically acceptable range and is preferably within a range of pH 4 to pH 8. For reference, a formulation example thereof is described below in the ~~section of~~ Example section. However, the formulation ~~example~~ examples ~~never limits~~ do not limit the scope of the invention.

The doses of Rho kinase inhibitor and prostaglandin can be determined depending on the ~~symptom~~ symptoms and age of the patients, the dosage form, the administration route and the like. The case of instillation is briefly described below. The dose of the Rho kinase inhibitor varies depending on the drug type. The Rho kinase inhibitor can be administered generally within 0.025 to 10,000 μg daily from once to several times a day. The dose can be appropriately raised or lowered depending on the age and ~~symptom~~ symptoms of the patients and the like.

The dose of prostaglandin varies depending on the type of prostaglandin ~~type~~. The usual daily dose is within a range of 0.1 to 1,000 μg, which can be administered from once to several times a day. More specifically, latanoprost and isopropyl unoprostone are generally administered at a daily dose of 1 to 5 μg and a daily dose of 30 to 300 μg, respectively. Depending on

the age and ~~symptom~~ symptoms of the patients and the like, the doses are varied. Based on similar standards, the doses of the other prostaglandins can be determined.

These doses are also applicable to the administration of the combination of a Rho kinase inhibitor and a prostaglandin. In the case that a Rho kinase inhibitor and a prostaglandin are to be administrated in one formulation, the formulation should be prepared by selecting the mixing ratio of the two drugs appropriately so that their daily doses might not ~~excess~~ exceed each dose of the separate drugs. The mixed formulation can be administered from once to several times daily.

Brief Description of the Drawings

Fig. 1 is a graph showing the changes of intraocular pressure with time in respective administration groups. The intraocular pressure is expressed ~~in~~ as a change from an initial intraocular pressure. □ represents a Compound A ((R)-(+)-N-(1H-pyrrolo[2,3-b]pyridine-4-yl)-4-(1-aminoethyl)-benzamide dihydrochloride) and isopropyl unoprostone combination administration group, ■ represents a single administration group of Compound A, □ represents a single administration group of isopropyl unoprostone, and ○ represents a control group.

Fig. 2 is a graph showing the changes of intraocular pressure with time in respective administration groups. The intraocular pressure is expressed ~~in~~ as a change from an initial intraocular pressure. □ represents a Compound B (1-(5-isoquinolinesulfonyl)homopiperazine dihydrochloride) and isopropyl unoprostone combination administration group, ■ represents a single

administration group of Compound B, □ represents a single administration group of isopropyl unoprostone, and ○ represents a control group.

Fig. 3 is a graph showing the changes of intraocular pressure with time in respective administration groups. The intraocular pressure is expressed ~~in~~ as a change from an initial intraocular pressure. □ represents a Compound A and latanoprost combination administration group, ■ represents a single administration group of Compound A, □ represents a single administration group of latanoprost, and ○ represents a control group.

Fig. 4 is a graph showing the changes of intraocular pressure with time in respective administration groups. The intraocular pressure is expressed ~~in~~ as a change from an initial intraocular pressure. □ represents a Compound B and latanoprost combination administration group, ■ represents a single administration group of Compound B, □ represents a single administration group of latanoprost, and ○ represents a control group.

Best Mode for Carrying out the Invention

A formulation example and pharmacological tests are shown in the following Examples. The Examples are for better understanding of the invention, but ~~never limits~~ do not limit the scope of the invention.

Examples

Formulation Example

A general formulation example of an ophthalmic solution comprising a Rho kinase inhibitor ((R)-(+)-N-(1H-pyrrolo[2,3-b]-pyridin-4-yl)-4-(1-aminoethyl)benzamide dihydrochloride) and a prostaglandin (isopropyl unoprostone) in the present invention is shown below.

Ophthalmic solution (in 100mL)

(R)-(+)-N-(1H-Pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide dihydrochloride	0.3 g
Isopropyl unoprostone	0.06 g
Boric acid	0.2 g
Concentrated glycerin	0.25 g
Benzalkonium chloride	0.005 g
Diluted hydrochloric acid	quantum sufficient
Sodium hydroxide	quantum sufficient
Purified water	quantum sufficient

Ophthalmic solutions having desired combinations and desired concentrations can be prepared by changing the kinds and amounts of the Rho kinase inhibitor and the prostaglandin and by appropriately changing the amounts of the additives.

Pharmacological tests

So as to study the utility of the combination of a Rho kinase inhibitor and a prostaglandin, ~~they~~ a Rho kinase inhibitor and a prostaglandin were administered to Japanese white rabbits (strain: JW, sex: male) or cynomolgus monkeys (Macaca fascicularis, sex: male), ~~examining~~ and the ~~effect~~ effects on reducing intraocular pressure were examined. (R)-(+)-N-(1H-Pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)-benzamide dihydrochloride [Compound A] or 1-(5-isoquinolinesulfonyl)homopiperazine dihydrochloride [Compound B] was used as the Rho kinase inhibitor. Isopropyl unoprostone or latanoprost was used

as the prostaglandin.

Preparation of test compound solutions

1. Preparation of Rho kinase inhibitor solutions

The Rho kinase inhibitor was dissolved in physiological saline, and then sodium hydroxide was added to the solution to neutralize it (pH 6.0 to 7.0) to thereby prepare Rho kinase inhibitor solutions having desired concentrations.

2. Preparation of prostaglandin solutions

A commercially available isopropyl unoprostone ophthalmic solution (trade name: Rescula ophthalmic solution) or a commercially available latanoprost ophthalmic solution (trade name: Xalatan ophthalmic solution) was used as it was, or was diluted with physiological saline to prepare prostaglandin solutions having desired concentrations.

Method of test

~~Administering the combination of the Rho kinase inhibitor and prostaglandin, the~~ The effect on reducing intraocular pressure by administering the combination of a Rho kinase inhibitor and a prostaglandin was studied. As a reference, ~~administering~~ the Rho kinase inhibitor was administered singly or the prostaglandin was administered singly, and the effect on reducing intraocular pressure was also studied. As a control, only a vehicle (physiological saline) was administered. As experimental animals, Japanese white rabbits (strain: JW, sex: male) or cynomolgus monkeys (sex: male) were used.

Method of administration and method of measurement

1. Administration of the combination of a Rho kinase inhibitor and a prostaglandin

1) One drop of a 0.4% oxybuprocaine hydrochloride ophthalmic solution was instilled into both eyes of each experimental animal to topically anesthetize ~~it topically~~ the eyes.

2) Intraocular pressure was measured immediately before administering the test compound solution, and the intraocular pressure was referred to as initial intraocular pressure.

3) The Rho kinase inhibitor solution was instilled into one eye of each experimental animal (the other eye was not treated). Since it is impossible to instill the prostaglandin solution at the same time, after a short period (about five minutes), the prostaglandin solution was instilled into the same eye.

4) Two, four, six and eight hours after instilling the Rho kinase inhibitor solution, one drop of the 0.4% oxybuprocaine hydrochloride ophthalmic solution was instilled into both eyes to topically anesthetize ~~it topically~~ the eyes. Then the intraocular pressure was measured three times to obtain the average of three measurements.

In Test 2 shown in the following Tests 1-4, intraocular pressure was measured after two, four and six hours.

2. Administration of a Rho kinase inhibitor alone

Each test was carried out in the same manner as in the above-mentioned combination administration test, except that the prostaglandin solution was replaced with physiological saline.

3. Administration of a prostaglandin alone

Each test was carried out in the same manner as in the above-mentioned combination administration test, except that the Rho kinase inhibitor solution was replaced with physiological saline.

4. Control

Each test was carried out in the same manner as in the above-mentioned combination administration test, except that the Rho kinase inhibitor solution and the prostaglandin solution were replaced with physiological saline.

Tests 1 to 4

The Rho kinase inhibitor solutions, the prostaglandin solutions and the experimental animals to be used in respective tests are shown in Table 1.

Tests 1 to 4 were carried out according to the above-mentioned method of test, and method of administration and method of measurement.

Table 1

	Rho kinase inhibitor solutions	Prostaglandin solutions	Experimental animals
Test 1	0.3% Compound A solution (50 μl)	0.06% Isopropyl unoprostone solution (50 μl)	Rabbit (four rabbits per group)
Test 2	1% Compound B solution (50 μl)	0.06% Isopropyl unoprostone solution (50 μl)	Rabbit (five rabbits per group)
Test 3	0.1% Compound A solution (20 μl)	0.005% Latanoprost solution (20 μl)	Cynomolgus monkey (three monkeys per group)
Test 4	1% Compound B solution (20 μl)	0.005% Latanoprost solution (20 μl)	Cynomolgus monkey (three monkeys per group)

Results and consideration

~~Results~~ The results of Test 1, the results of Test 2, the results of Test 3 and the results of Test 4 are shown in Figs. 1, 2, 3 and 4, respectively. Intraocular pressure is expressed in each Test as a change from an initial intraocular pressure.

As apparent from Figs. 1 to 4, all the Rho kinase inhibitor and prostaglandin combination groups exhibited excellent actions of reducing intraocular pressure compared with administration groups of each drug alone, (namely the Rho kinase inhibitor administration groups or the prostaglandin administration groups~~,~~) and exhibited improvement of the persistence of the actions. The above-mentioned results show that a stronger reducing effect on intraocular pressure and/or improvement of the persistence of the actions is obtained by combining ~~the~~ a Rho kinase inhibitor with a ~~prostaglandins~~ prostaglandin.

Industrial Applicability

An action ~~on~~ of reducing intraocular pressure is increased and/or persistence of the action is improved by administering to the eyes of a patient a Rho kinase inhibitor in combination with a prostaglandin ~~to eyes~~. Accordingly, the combination is useful as a therapeutic agent for glaucoma.